T. Lidsley.

Medical Research Council
War Memorandum No. 9

THE DETERMINATION OF BLOOD GROUPS

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INTRODUCTION

The disastrous results which may follow transfusions of incompatible blood, due to faulty blood group determination, are too well known to need emphasis. There is, however, less widespread knowledge of the technical principles which must be strictly adhered to if such errors in blood grouping are to be avoided.

During the last three years, blood group determinations have been made in Britain on an extensive scale, and the experience gained suggests that certain procedures, rigidly followed, will reduce possible errors in grouping to a minimum. The objects of the present memorandum are:—

(1) To describe methods which, in trained hands, have given satisfactory results.
(2) To recommend certain methods.
(3) To analyse the possible sources of error inherent in all group determinations. This entails a consideration of the essential qualities of Test Sera.

It is recommended that one or other of the methods proposed below should now be followed by all Transfusion Services, without the introduction of personal modifications by less experienced workers.

A note on the Rh factor is included for the sake of completeness, and methods of detecting Rh agglutinogens and agglutinins are described.

PRINCIPLES OF DIAGNOSIS OF THE ABO BLOOD GROUPS

The determination of blood groups is dependent upon a knowledge of the agglutinogen and agglutinin content of human blood. There are two main agglutinogens (A and B) which may occur either singly or together in human red cells, or be absent therefrom; and there are two corresponding agglutinins (anti-A or alpha and anti-B or beta) which occur in human sera, as indicated in Table I below.

Table I

Groups	*Agglutinogens in red cells*	*Agglutinins in serum*
AB	A and B	Neither
A	A	beta (anti-B)
B	B	alpha (anti-A)
O	Neither	alpha and beta

Haemagglutination occurs whenever homologous agglutinin and agglutinogen are in contact. It is therefore a biological axiom that homologous agglutinin and agglutinogen cannot exist together in the same blood.

It has long been common practice, when determining blood groups, to test unknown cells with known anti-A and anti-B serum, containing the alpha and beta agglutinins respectively. If agglutination occurs with both sera, the cells must contain both A and B agglutinogens, and therefore belong to group AB: if agglutination occurs only with alpha or anti-A serum (from a group B donor) the red cells contain A agglutinogen and must belong to group A: if it occurs with beta or anti-B serum (from a group A donor) the cells contain B agglutinogen and must belong to Group B: and if it does not occur with either serum, the red cells contain neither agglutinogen, and therefore belong to Group O.

TABLE II

Agglutinogen in cells	*" A " serum or anti-B (containing beta agglutinin)*	*" B " serum or anti-A (containing alpha agglutinin)*
AB	+	+
A	−	+
B	+	−
O	−	−

It is now recognised, however, that more reliable results are obtained when both serum and cells are tested, the dual test providing a check on technical and clerical errors. It is certain that if the serum were regularly examined for agglutinins, as well as the cells for agglutinogens, most blood grouping errors, both technical and clerical, would be prevented.

The Sub-Groups of A

The relatively simple statement that two agglutinogens (A and B, respectively) may be present in human red cells requires amplification, since there are two kinds of A agglutinogen, and bloods of sub-groups A_1 and A_1B and A_2 and A_2B occur. The chief importance of recognising this sub-division lies in the fact that the cells of A_2, and particularly A_2B, bloods react more weakly than those of A_1 and A_1B bloods with anti-A test sera; they may, indeed, react so weakly that the reactions are not detected at all unless test sera are used which are known to give satisfactory reactions with A_2 and A_2B cells.

From the practical point of view, the main significance of these sub-groups lies in the need to avoid the erroneous classification of bloods of sub-groups A_2 and A_2B as O and B, respectively, owing to failure to detect the A_2 factor. Approximately four out of five group A and AB bloods belong to the sub-groups A_1 and A_1B, and the remainder to the sub-groups A_2 and A_2B.

When transfusions are given to recipients of groups A and AB, it is not necessary to use blood of the appropriate sub-group, except in rare cases in which the recipient has been sensitised by a previous transfusion (see p. 5).

Nearly all anti-A sera (from group B and group O donors) contain the agglutinins alpha and $alpha_1$: alpha reacts with all the sub-groups A_1, A_2, A_1B and A_2B, while $alpha_1$ reacts only with the sub-groups A_1 and A_1B and *not* with A_2 and A_2B. To test for the sub-groups, $alpha_1$ is obtained free from alpha by absorbing a group B serum, containing $alpha_1$ in good amount, with a suitable quantity of A_2 cells; after separation of the cells by centrifugation, the $alpha_1$ remains in the supernatant fluid.

There are two additional complications:—

(i) The sera of 1–2 per cent. of A_2 persons, and of approximately 25 per cent. of A_2B persons, contain the agglutinin $alpha_1$, demonstrable at room temperature.

(ii) There is a further agglutinin, anti-O, which reacts with all O cells and occurs spontaneously, but very rarely, in A_1, B and A_1B sera. Unfortunately, this agglutinin is often called $alpha_2$, because it reacts with 95 per cent. of A_2 cells. It is thought that the A_2 cells which react with this agglutinin do so because they are of the genotype A_2O, and that the A_2 cells which fail to react are of the genotype A_2A_2. That anti-O agglutinates A_2B cells only weakly, if at all, supports this view. At first sight, it might appear that the nomenclature and understanding of the sub-

groups would be simplified if the term "$alpha_2$" were used for the agglutinin which reacts with the sub-group A_2. This, however, would not, in fact, be logical, for the agglutinin in question (alpha) reacts also with A_1 bloods, and to name it "$alpha_2$" would suggest it was specific for A_2 cells, as $alpha_1$ is specific for those of A_1. The name "alpha" applied to the agglutinin which reacts with both A_1 and A_2 sub-groups makes no suggestion that it is specific for either of them, and indeed indicates that it reacts with both kinds of A cells. Moreover, the term "$alpha_2$" has long been used by authors to denote the agglutinin anti-O, and though this is regrettable, it has to be borne in mind by a person seeking to understand the literature of blood grouping. To change the meaning of the term "$alpha_2$" would be to make confusion worse confounded, for it would result in the same name being applicable to two different agglutinins.

The extra agglutinins associated with the sub-groups are in most cases present in only small amounts, and are more active in the cold than at room temperatures. At a first transfusion they are unlikely to give rise to reactions due to incompatibility between the cells of the donor and the serum of the recipient. Such a first transfusion may, however, stimulate an increase in the titre of circulating extra agglutinin, and at subsequent transfusions the risk of incompatibility will therefore be increased. In actual practice, reactions due to such agglutinins, even in repeated transfusions, appear to be rare.

TABLE III

Group and Sub-group	*Reacts with agglutinins*	*Agglutinins usually occurring in serum*	*Agglutinins (possible extra)*
A_1	$alpha_1$ and alpha	anti-B (beta)	anti-O ($alpha_2$) (very rare) reacts with 95 per cent. of A_2 and all O cells
A_2	alpha (always) anti-O ($alpha_2$) (95 per cent. of cases)	anti-B (beta)	$alpha_1$ (1–2 per cent.) reacts with A_1 and A_1B cells
A_1B	$alpha_1$, alpha and beta	None	anti-O ($alpha_2$) (very rare) reacts with 95 per cent. of A_2 and all O cells
A_2B	alpha and beta: seldom if ever, with anti-O	None	$alpha_1$ (25–30 per cent.) reacts with A_1 and A_1B cells
B	beta	anti-A i.e. alpha & $alpha_1$	anti-O ($alpha_2$) (very rare) reacts with 95 cent. of A_2 and all O cells
O	anti-O ($alpha_2$)	anti-A (i.e. alpha & $alpha_1$) anti-B (beta)	None

METHODS

1. Tube Method.

The blood is examined both for agglutinogens in the cells and for agglutinins in the serum.

(*a*) *Test for agglutinogens in the cells:*—Test-tubes, $2'' \times \frac{1}{4}''$, preferably without lips, are placed in the first, second, fourth and fifth 10-hole rows of a fifty-hole wooden block (obtainable from R. B. Turner & Co., 9 Eagle Street, London, W.C.1); the middle row is not used.

High titre anti-A serum is placed in the tubes of the first and fourth rows, anti-B in the second and fifth rows. Red cells from a sample of blood to be tested are added to the first tubes in the first and second rows, cells from another sample to the second tubes in the same rows, and so on, so that by using the forty tubes in the block, red cells from twenty samples of blood can be tested against anti-A and anti-B sera.

Each tube receives a volume of serum, a volume of red cell suspension and, to avoid false agglutination, a volume of saline (as recommended by Landsteiner). To save time, the serum is diluted in bulk with an equal volume of saline. A Pasteur

pipette, graduated at 0·04 c.c. and 0·08 c.c., is filled to the upper mark and delivers 0·08 c.c. of the serum-saline mixture to the appropriate tubes. Later the pipette, after rinsing with water and saline, is filled to the 0·08 c.c. mark with red cell suspension, obtained by bleeding into 3·0 per cent. sodium citrate solution (or recovered from the blood clot) and adjusted to the required strength of about 5 per cent. of whole blood, as judged by eye from previous experience; 0·04 c.c. of the suspension is delivered into the anti-A and 0·04 c.c. into the anti-B tube.

As controls, cells of known groups A, B and O are put up in the same way. The tubes are shaken and capped with cylindrical glass caps, and left at room temperature for two hours. The caps exclude extraneous matter, prevent evaporation, and are almost indispensable as place-markers in reading.

Reading: After removal of its cap, each tube is picked up, and the cell deposit is dispersed from the bottom of the tube by flicking with a finger. The contents of every tube which does not show undoubted agglutinates are examined microscopically. This is done by placing a drop of the mixed contents on a slide, spreading it evenly by means of the stem of the Pasteur pipette, and reading under a low-power microscope. The strength of the reaction is scored as follows:—

C (complete) ..	Clumps of cells in clear surrounding fluid, visible to naked eye.
V (visual)	Rather weaker clumping, distinctly visible but with surrounding fluid not clear.
++	Very big clumps under microscope.
+	Not such big clumps.
(+)	Less than +, clumps of 8–12 cells.
W (weak)	Weak reactions with uniform distribution of small clumps of 4–6 cells.
?	Uneven distribution of cells, with no very definite clumps.

In tests of the cells for agglutinogens, a reaction less than + is very unlikely to be a true one and a retest should be made: the check given by testing the serum for agglutinins should prevent a wrong diagnosis.

In tests for agglutinins in serum, small reactions are quite frequent, and in infants about 50 per cent. of the agglutinins expected are not found. A "W" reaction would be taken as evidence of a true reaction; a "?" would require a retest; and in cases where the expected agglutinins are not disclosed after retest, a diagnosis has to be based on the agglutinogen content of the cells.

When a large number of specimens is to be examined, it is best to have one worker reading the macroscopical reactions and preparing the slides, and another worker using the microscope and recording all readings. A pair of experienced workers can read the agglutinogen tests on a hundred samples in twenty minutes, and the agglutinin tests in just under fifteen minutes.

(*b*) *Tests for agglutinins in serum.*—The test is set up as before in pairs of tubes, each of which receives 0·04 c.c. of serum, only one filling of the Pasteur pipette up to the 0·08 c.c. marking being needed. To the first tube are added known A_1 cells, to the second known B cells. No bulk of serum can be diluted with saline in this case and the necessary dilution is attained by adding to each tube 0·08 c.c. of appropriate red cell suspension, diluted to half the strength of the suspensions used in testing for the agglutinogens.

Further tests.

(i) *Weak "A" reactions in group AB.*—Particular care must be taken in accepting the diagnosis of group B, since the reactions with anti-A serum (B serum) are often markedly weaker when A occurs with B in group AB than when it occurs alone in group A; the B is then partially dominant to the A and obscures it. Group AB cells may therefore be falsely diagnosed as group B cells. This is particularly true in the case of the sub-group A_2B (see p. 4), in which the reactions given by the A agglutinogen are always weak. The examination of the serum for agglutinins does not necessarily serve as a safe check in this case, since, as shown in Table III, the serum from quite a number of A_2B persons contains the agglutinin alpha$_1$, which

reacts with A_1 but not with A_2 cells. As A_1 cells are used to check the serum, agglutination may occur and the diagnosis of group B cells be apparently confirmed. The mistake in diagnosis is, however, unlikely to arise if (as suggested on p. 11) anti-A serum is used which has been tested for its capacity to react with known A_2B cells. As an additional check, the serum of every blood sample which has been shown by determination of its agglutinogen and agglutinin contents to belong to group B is tested against known A_2 cells. If there is a reaction, it is clear that the serum must contain the agglutinin alpha, which reacts with A_2 as well as with A_1 cells. Therefore, the serum cannot have come from an A_1B or an A_2B person and must have come from a group B person. The diagnosis that cells belong to group B should not be accepted unless the serum has been checked against A_2 cells and found to give a reaction.

Evidence of failure to detect A with anti-A serum by the technique described has not been found in a single case.

(ii) *Alpha$_1$ occurring in group A_2 and A_2B.*—As already indicated, the serum of a group A_2 or of a group A_2B subject may give a reaction with known A_1 cells, due to the presence of alpha$_1$ agglutinin. If the serum is then tested with known A_2 cells and no reaction takes place, it may be assumed to contain alpha$_1$. The alpha$_1$ agglutinin found in the serum of A_2 and A_2B persons is nearly always present in small amounts, and the reactions with A_1 cells are usually not pronounced.

Anomalies in results.—The testing of both the agglutinogen content of cells and the agglutinin content of serum serves as a check on possible errors in recording, and on technical mistakes.

In all anomalous cases, the tests are repeated with cells which have been recovered from the clot, washed and re-suspended in saline, and with a fresh portion of serum. A definite result is always obtained from the re-test, save in the very rare case where the sample of whole blood is in a very bad condition; then a fresh one must be obtained.

Other anomalies, however, do arise, due to:—

(*a*) *False positives.* With fresh cells and the tube technique, there should be no trouble with false positive reactions. Occasional specimens which are not in good condition may need re-testing.

(*b*) *Missing agglutinins.* The case of a serum expected to react with A_1 cells and failing to do so has not been met. On the other hand, a reaction expected with B cells quite commonly amounts to no more than a "?", or is absent altogether, due to a deficiency or complete absence of anti-B agglutinin. The serum and cells are re-tested, and if the expected agglutinin is not disclosed (as frequently happens with the sera of infants), the diagnosis has to be based on the reaction given by the red cells.

2. Tile Method

The general principle of this method is that red cells are mixed with anti-B and anti-A serum on a white opal glass tile. Agglutination then shows up clearly against the white background. There are two, alternative, procedures:—

(1) in which cells in the form of a suspension are used,
(2) in which undiluted blood is used.

In both cases, it is advisable, when possible, to test both cells and serum in making a group determination upon unknown blood. When single group determinations are being made, blood may be taken by venepuncture, so as to obtain sufficient for serum separation. When large numbers of donors are being enrolled, however, this is not always practicable, and cells alone are obtained, by taking a few drops of blood from a prick in the ear or finger into citrate-saline solution. For the initial grouping at the time of enrolment, therefore, group determinations may be made upon cells alone. At the time when the donor is bled a large amount, as for an actual transfusion, a sample is always taken for a Kahn or Wassermann test. Serum, obtained from this sample, may be tested for agglutinins and the red cells tested for agglutinogens.

It is important to test the sera to be used each day, by putting up controls against known cells. In cold weather, to minimise the possibility of minor degrees of

"cold" agglutination, it is wise to warm the tile by holding it under the warm water tap; it must then be wiped dry before use.

(A) Tile Method with Diluted Blood.

(*a*) *Testing unknown cells.*—The diluent used may be either sterile normal saline (0·85 per cent.) or a sterile 1 per cent. solution of sodium citrate in normal saline. The latter should be recently and accurately made up, to avoid damage to the red cells. If the solution is too weak, haemolysis takes place: if allowed to stand, it is likely to become infected and may give rise to the Thomsen phenomenon (see p. 14). It is convenient to work with a series of Wassermann tubes, each containing 1 c.c. of diluent, placed in a numbered rack. Sufficient blood is obtained from a puncture in the lobe of the ear to give a 3 per cent. suspension of red cells in each tube, as judged by eye and experience. The tube should be plugged with cotton wool, or corked, if grouping is not to be carried out at once. Grouping should not be delayed longer than 36 hours; during this period of waiting the tubes should be kept in a refrigerator at between 2° and 4° C. When a large number of group determinations are being made, it is essential that a strict routine should be followed by all workers in a given laboratory. Grouping is carried out for preference on a flat white opal glass tile. A drop (0·05 c.c.) of a high titre anti-B serum (from group A blood) is placed in the left-hand space, and an equal amount (with another pipette) of high titre anti-A serum (from group B blood) placed in an adjacent right-hand space. To each of these is added a drop (0·05 c.c.) of the red cell suspension, by means of a pipette or a platinum loop. This pipette is washed out after each blood sample, with tap water and then with normal saline. Care must be taken never to mix high titre sera by using the same pipette for both anti-A and anti-B serum, and never to touch one serum with a blood pipette before adding the second drop of blood to the second serum. If a nickel-chrome or platinum loop (diameter 2 mm.) is used, it must be flamed, and cooled by dipping in normal saline, between successive drops.

When the serum and blood mixtures have been made, the tiles are rocked gently and left to stand. After at least 10 minutes, and before 15 minutes have passed, the tiles are agitated more strongly. Agglutination, if present, can easily be seen and the group read off the tile. In any case of doubt, the mixture of blood and serum should be examined with a hand lens.

(*b*) *Testing unknown serum.*—The technique is the same as that described for testing unknown cells, save that 0·1 c.c. drops of unknown serum are placed on the opal glass tile, and to them are added 0·05 c.c. amounts of the appropriate known cells. The cells and serum are mixed by agitation, left to stand, and the result read as before. It is essential to have a larger quantity of serum than cell suspension, since the antibodies in the serum may be present in small amounts. When discrepancies occur, both cells and serum should be re-tested, and, if necessary, be examined by the tube method.

(B) Tile Method with Undiluted Blood.

Drops approximating to 0·05 c.c. (e.g. as delivered by pipette No. 21 S.G.) of each of the high titre anti-A and anti-B sera are placed on the tile, in areas labelled with a grease pencil, care being taken in assigning each serum to its appropriate space. A drop of blood is obtained by needle puncture of the finger or ear lobe of the person to be grouped, and a very small loopful transferred to and mixed with each of the stock sera, with a platinum or other wire standard size loop (diameter 2 mm.). To avoid any tendency to pseudo-agglutination, and the addition of excess agglutinogen, the amount of blood added is only sufficient to colour the mixture a light pink. The loop must be heated and cooled before each addition.

The circular area covered by each red cell-serum mixture should be 1½–2 cm. in diameter. The tile is gently agitated, so as to ensure adequate admixture of the red cells and sera; and provided the latter are potent, agglutination, if it is going to occur, will show macroscopically—usually within two minutes and always within four minutes. Temporary cessation of the agitation for a few seconds at a time

augments the speed and degree of agglutination. The use of a small hand lens of about × 10 magnification is helpful, especially in detecting agglutination of A_2 cells, but a microscope is not required.

Re-examination of the tile after the sera and cells have been standing for some time, e.g. 10 minutes, may reveal the appearance of false agglutination, owing to partial drying and rouleaux formation in a previously negative mixture. No significance should be attached to such findings.

Unknown sera are preferably tested against diluted red cells. The titre of the agglutinins in such sera may be very low (as compared with that in stock grouping sera), and a longer period must of necessity elapse before reading. The use of a dilute suspension of stock cells in citrate saline is advantageous in counteracting drying and consequent rouleaux formation during this longer period.

3. Relative Merits of the Methods Described

It is generally agreed that the most reliable technique for group determination is the tube method, more especially in the case of the weak A reactions in A_2 and A_2B sub-groups. It may, however, take a slightly longer time, and it requires more glass-ware than the tile method, and this under war conditions may be a practical objection to its use in centres where group determinations are carried out on a large scale. A tile technique may therefore be employed in such circumstances. If undiluted blood is used, the following difficulties may be met:—

(1) The serum concentration in the mixture may be high and conditions favouring pseudo-agglutination may be produced.

(2) There is a risk of adding excess agglutinogen and so obtaining a false negative; this is of particular importance in the case of the weaker forms of A. It is claimed by workers experienced in the method that such difficulties are theoretical rather than practical. Their existence, however, cannot de denied, and it is felt, therefore, that undiluted blood should not be employed except by laboratory workers of long experience.

Recommendation 1.—The tube method is the most reliable, and where this is found impracticable, a tile technique, using diluted blood, is recommended. Undiluted blood should not be used unless the laboratory workers have had long experience of the technique.

Recommendation 2.—In the case of blood issued for transfusion, cells should be examined for agglutinogens, and serum for agglutinins. The double check is essential.

DIRECT MATCHING

Direct matching of the donor's cells against the recipient's serum before transfusion is extremely important, especially when blood of groups other than O are used. It must not, however, be considered a substitute for blood grouping tests, and these should always be made when grouping sera are available. In fact, under normal conditions both blood group determinations and direct matching should be carried out. Direct matching should be omitted only in an acute emergency—when delay of a few minutes in giving the transfusion may endanger the patient's life—or when large numbers of transfusions must be given by limited personnel working under emergency conditions. Under such conditions, it may be possible to start a serum or plasma transfusion and, while this is in progress, to perform direct matching and blood grouping tests before giving whole blood. On the other hand, circumstances must obviously arise when direct matching is the only procedure possible—for instance, when blood transfusion is indicated as an urgent therapeutic measure and adequate grouping sera are not available. In these circumstances, direct matching can alone be relied upon. The serum of the recipient should then be tested against the cells of possible donors, by the technique described below. Those donors whose cells are not agglutinated by the recipient's serum may be considered compatible.

When, for any reason, direct matching cannot be performed, known Group O blood should be given.

Technique of Direct Matching.

The technique of direct matching is fundamentally the same as that already described. Instead of known anti-A and anti-B serum, the serum of the recipient is used. The donor's cells should preferably be diluted with saline or citrate saline. If undiluted blood is used, the same difficulties as arise in the determination of the ABO blood groups with undiluted cells are likely to be encountered, namely, lowered sensitivity and increased liability to rouleaux formation. If rouleaux formation is suspected, a drop of saline should be added to the cell-serum mixture. In the case of stored blood, the bottle should be inverted to mix the cells and supernatant fluid, and a few drops of the blood, removed by means of a sterile pipette, be added to saline or citrate saline. Direct matching is then done in the usual way. A pilot tube attached to the bottle, and filled at the same time as the bottle, has not, in practice, proved a satisfactory method of providing cells for direct matching; infection is liable to occur in the pilot tube and a false positive reaction (the Thomsen phenomenon) may result. If the bottle is opened and not used for any reason, it must be discarded, to avoid any possible risk of using infected blood. Sufficient serum can be obtained from a few drops of blood taken from the ear and allowed to clot in a Widal tube, or from 0·5–1 c.c. of blood taken from a vein with a syringe and fine hypodermic needle and put into a small test-tube. If necessary, plasma may be used instead of serum; 8 drops of blood are added to 3 drops of 1 per cent. citrate solution and allowed to stand until sedimentation occurs, when the plasma may be pipetted off and used in the same way as serum. Twice as much serum or plasma as cell suspension should be used. Cases where the presence of anti-Rh agglutinins is suspected need special consideration (see p. 17).

Errors in Direct Matching.

As in ordinary blood grouping tests, errors may arise in direct matching. These usually result from false positive reactions, and it is wrongly concluded that the bloods of donor and recipient are incompatible. Such false positives are commonly due to pseudo-agglutination or rouleaux formation, and more rarely to cold agglutination depending upon the presence of specific or non-specific agglutinins. These phenomena are described in detail elsewhere (see pp. 13 and 14). Rouleaux formation can almost invariably be prevented by the addition of a drop of saline, or, preferably, by using diluted donor blood in the first place. Cold agglutination is prevented by warming the tile or slide on which the direct matching is carried out. To minimise risk of these errors, direct matching should be carried out, whenever possible, by trained laboratory personnel.

ESSENTIAL PROPERTIES OF TEST SERA

Serum which is to be used for blood-grouping must fulfil certain conditions, and be handled with certain definite precautions.

1. *High Titre.*—Only high titre sera, preferably checked against the Medical Research Council Standard, should be used.

The issue of these standard sera should obviate the difficulty of obtaining any agreed method of expressing the titre of serum. In the past, this difficulty has often resulted in failure to maintain a minimum level, even among those workers who recognise that a definite level of titre is necessary for typing serum.

The standard sera are in the form of powder obtained from drying 1 c.c. of fluid serum. Immediately before use, 1 c.c. of sterile distilled water should be added, the powder dissolved and the resulting serum well mixed. It is probably advisable to use the serum only on the day it is reconstituted. No serum should be chosen which does not react as strongly as the standard. The strength should be estimated by some method of titration which determines the highest dilution of the serum still causing agglutination. It is not enough to compare, without titration, the reactions given by the standard and the serum under test. Unless a serum has a satisfactory titration value to begin with, it will probably lose strength fairly rapidly.

2. *Capacity to react with A_2 and A_2B cells.*—As mentioned on p. 6, some anti-A sera, which are of good strength when titrated with A_1 cells, react only poorly with the weaker form of A found in the sub-groups A_2 and A_2B. Such sera will often fail to reveal the A factor in the sub-group A_2B, and the cells may be wrongly diagnosed as belonging to group B. Sometimes A_2 is missed and diagnosed as O. No anti-A serum should be used unless it behaves as well as the standard with A_2B cells.

A description of a method of titrating anti-A serum in order to determine its capacity to react with A_2B cells, together with protocols illustrating the results of such a titration, is given below.

It should be determined that, with the technique to be used, an anti-A serum gives no sort of a reaction with B or O cells; that an anti-B serum does not react with A or O cells; and that an anti-A serum gives a satisfactory result with A_2B cells.

3. *Absence of Cold Agglutinins.*—Sera should be chosen which are free from cold agglutinins. These can be removed to a considerable extent by resuspending the cells from around the clot after the sample has attained refrigerator temperature, allowing the cells to resettle and separating the serum in the cold.

4. *Absence of a tendency to cause Rouleaux Formation.*—Certain sera appear to favour rouleaux formation. These should be excluded from use as test sera.

5. *Freedom from Fat.*—Fat in a grouping serum tends to accelerate loss of strength, and gives the serum a doubtful appearance, suggestive of bacterial contamination. Donors from whose blood grouping serum is to be prepared should, preferably, take no fatty food for some hours before being bled.

A METHOD OF TITRATING TEST SERA

Precipitin tubes, $2'' \times \frac{1}{4}''$, are used, and equal volumes of the reagents are measured by a Pasteur pipette, marked to deliver about 0·06 c.c., with an india-rubber teat. The strength of the cell suspension is kept constant* (about 5 per cent. of whole blood, as judged by eye and experience), the serum dilutions being increased two-fold at each stage. As diluent, a 0·85 per cent. solution of sodium chloride in distilled water is used. The serum-cell mixtures are shaken to yield even suspensions, left on the bench for two hours, and then read: the highest dilution of serum giving a reaction of at least " W " is taken as the end-point. The numerical titre of a serum varies markedly with the method of titration used.

TITRATION OF TWO ANTI-A SERA

(The system of scoring used has been described on p. 6.)

	Serum 1				*Serum* 2			
Serum dilution†	A_1	A_2	A_1B	A_2B	A_1	A_2	A_1B	A_2B
1/1	C	V	C	+	C	C	C	++
1/2	C	++	C	(+)	C	V	C	++
1/4	C	++	V	W	C	V	V	+
1/8	V	+	V	−	V	++	V	+
1/16	++	(+)	++		V	(+)	++	W
1/32	++	−	W		++	−	++	−
1/64	+				+		W	
1/128	(+)				W		−	
1/256	−				−			

† Many workers use this notation, although, strictly speaking, the denominator should be doubled, since dilution to this degree is caused by the addition of the cell suspension.

It will be seen that, although both sera behave very similarly with A_1 cells, number 1 is much the poorer with A_2 and A_2B cells.

STORAGE OF TEST SERA

(1) *Temperature.*—Strength deteriorates if the serum is kept at room temperature. It should therefore be kept, preferably frozen solid, in an ice chest. If kept in bulk in this manner, its strength is preserved almost indefinitely. If kept in bulk at between 2° and 4° C., its strength is preserved for a variable period.

* Cells for the purpose of titration should be either pooled cells from a minimum of six donors or the cells of a person who is regularly used as a standard.

(2) *Fluid.*—It has been common practice in this country to use liquid serum for group determinations. Serum should be stored in bottles or ampoules which contain amounts suited to the particular needs of the workers who are to use them; a choice of different sizes will be an advantage. If serum is to be frozen solid, bottles or tubes are better than ampoules, and they should be only $\frac{2}{3}$ full. A frequent small issue of supplies is more satisfactory than a single large issue, unless it is known that satisfactory arrangements for refrigeration are available. Questions of sterility, complete sealing, avoidance of overheating, and the selection of suitable glass, render it difficult to put up as satisfactory a product in capillary tubes as when serum is stored in bulk.

(3) *Asepsis.*—No antiseptic should be added to test sera. Sterility is important, because if bacterial contamination occurs, the titre may fall or the serum may give false positive reactions. Serum should, therefore, be put up in such amounts as will avoid the need to use the same container on many occasions. No bottle, once opened, should be used for longer than a week. Serum should always be withdrawn from the bottle with a sterile pipette. For this purpose, a drum of sterile Pasteur pipettes should be available.

(4) *Dried Grouping Serum.*—Grouping serum needs to be completely dry if it is to retain its properties in the dry state. It should be dried by a low-temperature low-pressure process, should be rendered completely anhydrous and should be efficiently sealed in nitrogen, to ensure good preservation of the titre. The dried product will keep indefinitely without refrigeration, and is therefore recommended for places where no refrigerator is available. For use, it is necessary only to add the correct amount of distilled water.

POSSIBLE SOURCES OF ERROR IN BLOOD GROUP DETERMINATIONS

Errors in blood group determinations may be (A) technical, and (B) clerical, in origin.

(A) Technical Sources of Error may lead to either false negative or false positive results.

False negative results may be due to:—

(1) Failure to use high titre sera.
(2) Failure to use an anti-A serum capable of reacting with A_2 and A_2B cells.
(3) Failure to recognise the time factor.
(4) The use of infected serum.

False positive results may be due to:—

(1) Pseudo-agglutination or rouleaux formation, due to (*a*) delayed reading or (*b*) rarer causes.
(2) Cold agglutination.
(3) The use of infected cell suspensions (the Thomsen phenomenon).
(4) The use of infected serum.

False Negatives

(1) *Failure to use high titre sera.*—The issue of standard dried sera of high titre should theoretically obviate the danger of using a weak serum. It must be borne in mind, however, that, unless the serum is stored with due regard to the precautions outlined above, there may be definite deterioration in antibody titre.

(2) *Failure to use serum capable of disclosing the weak A factor in sub-groups A_2 and A_2B.*—Some anti-A sera which are of high titre when tested against A_1 cells react very poorly with A_2B cells, and not well with A_2 cells: with such a serum, A_2B cells are often diagnosed as B, and occasionally A_2 cells as O.

(3) *Failure to recognise the time factor.*—When diluted cell suspensions are used, the results should not be read before 10 minutes have elapsed, or false negatives may be reported.

(4) *Infected serum.*—If bacterial contamination of the serum occurs, the titre may fall and false negative results be obtained.

(1) *Pseudo-agglutination or Rouleaux Formation* rarely gives trouble to an experienced worker. It is favoured by a high concentration of serum and by increase of temperature. If the serum is diluted 1 in 2, or 1 in 3 with normal saline, the rouleaux formation tends to disappear, whilst true agglutination will persist. Rouleaux formation is not true agglutination, for it is not due to an absorbable agglutinin.

The commonest cause of mistakes due to rouleaux formation is delay in reading the result when using a tile method. In certain pathological conditions, intense rouleaux formation may make the determination of the blood group difficult. Such rouleaux formation is rarely, if ever, seen with the serum of healthy people, but it is a common source of error in patients with septic conditions and high fever.

The statement, often made, that false and true agglutination are readily distinguishable under the microscope, is far from being invariably true: only in cases in which rouleaux formation is very obvious can the distinction be made with certainty.

Some grouping sera develop the property of agglutinating red blood cells of a group which they should not agglutinate. In most cases, a serum giving such non-specific reactions with cells of a certain group reacts with cells from only some, but not all, members of this group.

Usually, but not always, pseudo-reactions are very much less intense, and take much longer to appear, than true reactions: they often amount to no more than a "graininess," and nearly always disappear when a drop of saline solution is added to the cell-serum mixture. Reactions which are much weaker and tardier than those given by known control cells are most unlikely to be true positives. Since, however, the reaction between anti-A serum and some cells of the group AB may be very slight, a delayed and weak reaction with anti-A by cells which react well with anti-B serum may leave the worker in doubt as to the correct group. In such cases, it is advised that one volume (say about 0·1 c.c.) of anti-A serum, one volume of saline solution, and one volume of red cell suspension (about 4–5 per cent. whole blood) should be put into a small test tube. The contents of the tube should be mixed by shaking and left on the bench at room temperature for two hours; the tube should then be re-shaken and a drop of its contents examined for agglutinates under a low-power microscope.

(2) *Cold Agglutinins.*—Besides the iso-agglutinins already described, another class of agglutinins may occur in normal sera, which, because in most cases they act only at low temperatures, are called cold agglutinins. Occasionally, however, they are active at room, and very rarely at body temperatures. It may be noted that the usual iso-agglutinins anti-A and anti-B do react more strongly at lower temperatures, but they also react quite well at body temperature.

There are two kinds of cold agglutinins (*a*) Non-specific and (*b*) Specific.

(*a*) *Non-specific cold agglutinins.*—Most cold agglutinins are non-specific in their action and will agglutinate all human red cells, irrespective of group. As they also agglutinate the cells of the person in whose serum they occur, they are often called auto-agglutinins. Non-specific cold agglutinins which are active at room temperature lead to the following blood grouping difficulties:—

(i) *In grouping the red cells of a person whose serum contains auto-agglutinins active at room temperature.*—The unwashed red cells may be agglutinated regardless of the testing serum, owing to the presence of auto-agglutinins active at room temperature, and they may therefore be diagnosed incorrectly as of group AB. Unless the serum is also examined for iso-agglutinins, a false result is almost certain. When the serum is thus examined, it will agglutinate standard A and B cells and therefore appear to be the serum from a group O blood. When such anomalous results are obtained, the action of the cold agglutinin can usually be prevented from interfering with the proper testing of cells for iso-agglutinogens, and of serum for iso-agglutinins, by making the tests at 37° C.—preferably with cell suspensions and sera already brought to that temperature. Alternatively, to facilitate blood grouping in such cases, red cell suspensions free from auto-agglutinins can be obtained by washing the red cells with saline solution at 37° C. As all cold agglutinins are true agglutinins, they can be removed from serum by mixing it with red cells containing the appropriate

agglutinogen; this absorption, and the subsequent separation of the serum from the cells, should take place at refrigerator temperature, 0 — 5° C. Thus, non-specific agglutinins (auto-agglutinins) can be removed to a considerable extent by resuspending the cells from around their clot after the whole blood sample has attained refrigerator temperature, allowing the cells to settle again and separating the serum in the cold.

(ii) *In the direct matching of recipient's serum and the red cells of prospective donors.*—It may be found that a patient's serum agglutinates at room temperature the red cells of all of a series of prospective donors, even though these donors belong to the same group as the patient or to another compatible group. If the agglutination persists after dilution with saline solution, and if the recipient's own cells are also clumped by his own serum, a non-specific cold agglutinin (auto-agglutinin), active at room temperature, is present in the patient's serum. The tests should then be repeated at 37° C, preferably after the reagents have been brought up to that temperature, when the absence of clumping will confirm that the trouble has been due to a cold agglutinin, and show that the donors are really compatible.

(iii) *In anti-A and anti-B testing sera.*—Sera which contain additional agglutinins, active at the temperature at which grouping tests are to be made, are obviously unsuitable as testing sera. Non-specific agglutinins reacting with all human cells would almost certainly be detected at once if present in a serum intended for grouping tests.

(*b*) *Specific cold agglutinins.*—Specific cold agglutinins, acting on particular groups, or sub-groups, also occur. The two most frequently encountered are (1) those which agglutinate A_1 and A_1B cells ($alpha_1$) and (2) those which agglutinate all O and most A_2 cells (anti-O). Very rarely indeed, spontaneous agglutinins to the blood-cell agglutinogens M, N and P have been found in human sera, and these agglutinins tend to be more active at lower temperatures. When specific cold agglutinins are active at temperatures at which grouping tests are made, they lead to difficulties similar to those described in connection with non-specific agglutinins, save that they do not agglutinate all human bloods nor the cells of the donor of the serum. Sera cannot be rid of specific agglutinins by absorption reactions with their own cells: cells containing the appropriate iso-agglutinogen, and obtained from another donor, are needed.

Grouping difficulties due to either specific or non-specific cold agglutinins can be resolved by removing these agglutinins from sera and red cell suspensions as described above, and by performing tests at 37° C. To group some bloods, it may be necessary to adopt all these measures.

Non-specific cold agglutinins are liable to become more potent, and to react at room temperature, in certain pathological conditions, e.g. severe anaemias, liver disease and severe infections.

The characteristics of pseudo-agglutination, cold agglutination and true iso-agglutination are summarised in the table below, which is modified from one given by Landsteiner:—

DIFFERENTIATION OF PSEUDO-AGGLUTINATION, COLD AGGLUTINATION AND ISO-AGGLUTINATION

	Pseudo-agglutination	*Cold agglutination*	*Iso-agglutination*
Absorption of active principle	Not absorbable	Absorbable	Absorbable
Effect of temperature on tests	Not weaker, rather stronger at 37° C. than at low temperature	Occurs as a rule only at low temperature	Is little affected by changes of temperature from 0° to 37° C.
Effect of dilution	Is inactivated by slight dilution	Stands considerable dilution	Stands considerable dilution
Specificity	Non-specific	Usually non-specific	Group specific

(3) *Infected Cell Suspensions* (*The Thomsen Phenomenon*). Several types of contaminating bacteria are capable of causing haemagglutination. They may be present on the glassware or tubes used for the manipulation of blood. The reaction

may become apparent within 18 hours at 2° C., or within even shorter periods at room temperature. Agglutination then occurs because the cells themselves have become agglutinable by practically any normal human or animal serum. It is important, therefore, to use only fresh red cells or cells which have been stored for the shortest possible time in the refrigerator. In blood stored in the form of clot, the phenomenon is very rarely found. The extreme importance of this source of error has not hitherto been sufficiently appreciated, especially in pilot tubes attached to blood bottles issued from storage for cross-matching.

(4) *Infected Serum.*—The use of infected serum may give rise to false positive reactions.

(B) Clerical Errors can be avoided only by careful checking at every stage, by everyone concerned with the transfusion service. For instance, no bottle of blood should be issued from the blood bank unless the group on its label has been checked against the laboratory record book by the person issuing the blood.

THE Rh FACTOR

Human erythrocytes may contain, in addition to the A and B agglutinogens of Landsteiner, a variety of antigenic components. These other agglutinogens, of which M and N have until recently been the best known, are usually ignored by clinicians in the selection of blood donors, because the corresponding agglutinins occur only very rarely in human sera. Moreover, these agglutinogens very rarely stimulate the formation of immune agglutinins in man.

It has recently been shown that the red blood cells of 85* per cent. of human subjects, irrespective of their ABO group, contain a hitherto unrecognised agglutinogen, Rh; the term Rh is derived from the fact that a similar agglutinogen is found in the red blood cells of rhesus monkeys. The importance of this discovery lies in the fact that the remaining 15 per cent. of persons, whose red cells lack the Rh agglutinogen, are liable to form an antibody (agglutinin) against the agglutinogen if it is introduced into their circulation. This may occur in an " Rh negative " woman if she becomes pregnant with a foetus whose blood cells are " Rh positive," or it may happen in " Rh negative " persons of either sex when Rh positive blood is transfused. Anti-Rh agglutinins are formed only under one of these two conditions —that is to say, they do not occur naturally like the anti-A and anti-B agglutinins.

From the transfusion point of view, the chief significance of the formation of anti-Rh agglutinins by an Rh negative person, whether this occurs as a result of pregnancy or of transfusion, lies in the fact that a subsequent transfusion of Rh positive blood may lead to a severe haemolytic reaction, which may even be fatal. Since 85 per cent. of human bloods are Rh positive, blood which is selected only on the basis of ABO group compatibility is very likely to prove incompatible in these Rh negative subjects, and the only compatible donors for such cases are Rh negative persons of suitable ABO group.

It is of considerable clinical importance to be familiar with the types of case in which the formation of anti-Rh agglutinins should be suspected.

(A) The Relationship Between Iso-immunisation of the Mother to the Rh Factor and Erythroblastosis Foetalis in the Infant

In over 90 per cent. of cases in which the infant is affected with erythroblastosis foetalis, the mother is found to be Rh negative and the infant Rh positive. In such cases, the mother produces anti-Rh agglutinins during pregnancy, and it is the passage of these agglutinins from the maternal circulation into the foetal circulation which causes the destruction of the foetal erythrocytes. It must be emphasised that the manifestations of erythroblastosis foetalis are very varied. The infant may

* This figure is approximately correct for American and English white subjects: racial differences in distribution occur, as with other blood group factors.

be oedematous, jaundiced, or anaemic at birth, or may appear normal at birth and not develop jaundice or anaemia until later. Many affected infants are stillborn, often prematurely, and may be oedematous or have an oedematous placenta. On the other hand, the stillborn infant may be outwardly normal. In a case in which there is no other obvious cause for foetal morbidity, the possibility of erythroblastosis foetalis should always be kept in mind. If the mothers of such cases require transfusion, only known Rh negative blood of suitable ABO group should be used.

(B) Iso-immunisation of Persons of Either Sex to the Rh Factor following Transfusion

The transfusion, and particularly the repeated transfusion, of Rh positive blood to Rh negative recipients of either sex, is liable to lead to the development of Rh agglutinins in the recipient's circulation, and thus to be followed by the premature destruction of the donor blood cells. At first, this process may be entirely " silent "; alternatively, the only evidence of the increased rate of elimination of the transfused blood may be a failure of the recipient's haemoglobin concentration to increase following transfusion. If further transfusions of Rh positive blood are given, however, the reactions become increasingly severe, the destruction of the donor blood cells is more rapid and the recipient may become jaundiced or even develop haemoglobinuria. Sometimes, no immediate reaction to the transfusion is observed, but the recipient develops oliguria after transfusion and passes into a state of uraemia some days later. The occurrence of any of these signs, and particularly the increasingly unfavourable response to transfusion, should always suggest the possibility that the recipient has become immunised to the Rh factor, provided, of course, that the possibility of incompatibility due to faulty ABO grouping has been excluded.

Discussion.

Ideally, Rh negative persons should be transfused only with Rh negative blood. At present, however, this ideal may be regarded as impracticable, because of the technical difficulties of making Rh grouping tests on a large scale, and because of the greater administrative difficulty of keeping a sufficient number of Rh negative bloods in small banks, to serve the needs of all Rh negative recipients. Although the technique of making tests for Rh agglutinogens and agglutinins is described below, it must be emphasised that the interpretation of these tests often presents considerable difficulty, and, until the necessary specialised experience has been acquired, it is urged that the recommendations set out on p. 18 should be followed in such cases.

Tests for Rh Agglutinogens and Agglutinins

Sources of Test Serum.—Test serum may be obtained from two sources. Either an animal, preferably the guinea-pig, may be immunised by being given a course of injections of blood from the monkey, *Macacus rhesus*, or serum may be obtained from a human being who has become immunised to the Rh antigen. Animal sera have to be absorbed, since they contain other antibodies acting upon human erythrocytes; on the other hand, they can be produced at will and their reactions are more uniform than those of human sera. The advantage of using immune human sera is that in a favourable case a comparatively large amount of serum may be obtained with little trouble. Human sera containing immune anti-Rh agglutinins may, of course, also contain anti-A or anti-B agglutinins; these may either be absorbed with group A or B Rh negative blood, or they may be neutralised with appropriate human saliva. If such absorption or neutralisation is not carried out, such sera can obviously be used only to test red cells of the same group, or for cells of group O blood.

It will be seen that one of the difficulties in the detection of Rh agglutinogens, compared with the detection of the A and B agglutinogens, is that potent specific sera are difficult to obtain. Even with good sera, the reactions are usually weaker than the ordinary iso-agglutination reactions.

Testing of Erythrocytes for the Presence of the Rh Agglutinogen

The following technique, which follows closely that of Landsteiner and Wiener, is recommended. A suspension of the cells to be tested is prepared by adding one small drop (0·02 c.c.) of blood to 1 c.c. of saline; the final concentration should be approximately 1·0 per cent. in terms of blood sediment. One volume of this suspension is placed with one volume of the test serum in a small tube, 7 mm. in diameter. It is desirable to test cells with not less than three different sera, for reasons which will be given below. At the same time, known Rh positive and Rh negative cells should be put up with the same sera, to act as controls. The tubes should be left for at least one hour, and preferably for two hours, before being examined. With many sera, the reactions are quite satisfactory when the tubes are left at room temperature, but the reactions are usually stronger at 37° C., and unless the sera are known to give consistently good reactions at room temperature, the tubes should be placed in a water bath or incubator at 37° C.

After the tubes have stood for one to two hours, the sediment which has formed is examined, preferably with the help of a hand lens. When the reaction is negative, the sediment is found to be homogeneous and to have a clear-cut circular edge. When the reaction is positive, the sediment appears granular and the edge is serrated. When the tubes have been examined in this way, and the reactions scored, the sediment in each tube is *gently* withdrawn by means of a Pasteur pipette and spread very gently on a slide. Usually, definite clumping is seen in blood taken from the tubes giving macroscopic positives. Occasionally, however, only very small agglutinates are seen, despite a characteristically positive sediment. The recognition of the different sediment patterns is useful in giving an indication of the reaction to be expected, but in case of doubt it is advisable to rely upon the microscopical appearances. It must be emphasised that harsh treatment of the mixtures will often completely abolish the agglutination, so that no trace of clumping is seen when the cells are examined under the microscope. False negative reactions are thus one of the greatest difficulties, and this is one of the reasons for recommending the use of more than one serum for the tests.

When the reactions are weak, it may be very difficult to decide from the microscopical appearance whether or not the reaction is true agglutination. This is a further reason for the use of several different sera, and for carrying out control tests with known Rh positive and Rh negative cells. Whenever sufficiently potent sera can be obtained, it is desirable to add a volume of saline to the volume of serum and of cells, in order to abolish rouleaux formation.

Detection of Anti-Rh Agglutinins in Human Sera

When there is reason to suspect the presence of atypical agglutinins in a sample of human serum, the following procedure is recommended:—

The serum is tested against not less than two known Rh positive and two known Rh negative bloods, using the technique described above and carrying out the tests at room temperature and at 37° C. Since the sensitivity of different Rh positive cells varies very greatly, at least one of the Rh positive bloods should have been taken from a known good reactor.

Some sera containing anti-Rh agglutinins exhibit "zoning"; that is to say, the reactions given by the undiluted serum may be smaller than those given by certain dilutions of the serum. In occasional cases, the sera may fail altogether to react with some Rh positive bloods. For this reason, a serum suspected of containing anti-Rh agglutinins should be tested against several known Rh positive bloods or, alternatively, serial dilutions of the serum should be tested against not less than two Rh positive bloods.

It should be emphasised that anti-Rh agglutinins are immune bodies, and that their potency therefore varies greatly according to the time at which a sample is taken. Following the transfusion of Rh positive blood to a recipient whose serum contains anti-Rh agglutinins, the agglutinins may disappear completely from the circulation for a short time, and they may not become easily detectable again for,

say, 5 days after transfusion. Thereafter, their potency will increase and then wane again. These facts must be borne in mind when a search is made for anti-Rh agglutinins in human sera.

Recommendations

(1) Mothers of infants which manifest signs of erythroblastosis foetalis should not be transfused with blood unless known Rh negative blood of suitable ABO group is available. In cases urgently needing transfusion, plasma or serum should be used instead of whole blood.

(2) Recipients of either sex who, from their response to previous transfusions, are suspected of having become immunised to the Rh factor should receive no further whole blood transfusions (unless Rh negative blood of suitable ABO group is available), until the cause of the unfavourable response to the previous transfusion has been investigated.

(3) When transfusions are given to infants affected with erythroblastosis foetalis, blood from a group O, Rh negative donor should be used whenever possible. If no such donor is available, the mother's red cells, if of suitable ABO group, may be used, provided that facilities are available for washing them free of plasma. When this, too, is impracticable, and transfusion is urgent owing to the anaemic state of the child, a donor of group O, taken at random, may have to be used, although it should be realised that such blood will usually be Rh positive, and that its transfusion may produce a transient increase in jaundice in the infant. In these circumstances, it is better to seek a donor amongst the mother's relatives than to use the father or his relatives.

(4) Although, when the patient's serum contains anti-Rh agglutinins, it is often possible to select a compatible donor by testing the patient's serum against a number of different bloods of suitable ABO (but unknown Rh) group, using the technique recommended above, reliable results are very difficult to obtain without considerable experience, and it is strongly recommended that this method should not be attempted in practice by persons lacking such experience.

(5) In all these circumstances, it is recommended that contact should be made as soon as possible with the nearest Blood Depot or Regional Blood Transfusion Officer.

TRAINING OF PERSONNEL

All blood group determinations should be entrusted only to personnel who have had a sufficient period of training in a laboratory accustomed to such work. The necessity of employing trained personnel is emphasised, since there has hitherto been a tendency to consider that anyone can be taught to determine blood groups in a few minutes. Now that adequate donor panels are available throughout the country, it should be unnecessary for any mass grouping to be done by inexperienced workers. It is essential, if errors are to be avoided, that workers who are making blood group determinations should fully understand the underlying principles of the methods.

SUMMARY

(1) The principles governing the diagnosis of the ABO blood groups are discussed.
(2) Three methods of blood group determination are described, (*a*) the Tube Method, (*b*) the Tile Method, using diluted blood, (*c*) the Tile Method, using undiluted blood.
(3) It is recommended that blood issued for transfusion from the large centres should have group determinations checked on both cells and serum.
(4) Where possible, the Tube Method should be used: if this is impracticable, A Tile Method, using diluted blood, is advisable.
(5) The use of undiluted blood, except by laboratory workers of long experience, is not advised.

(6) Direct matching of the donor's red cells against the recipient's serum before transfusion is desirable when group O blood is to be given, and is essential when blood of any other group is to be given. When workers with sufficient laboratory experience to make this test are not available, group O blood may be used without direct matching. A pilot tube containing blood collected at the time of blood donation should *not* be used for matching.

(7) The essential properties of test sera are defined as follows: (i) high titre: (ii) capacity to react with A_2 and A_2B cells: (iii) the absence of cold agglutinins: (iv) the absence of rouleaux-forming properties: (v) the absence of excess fat: (vi) the absence of contamination.

(8) The possible sources of error in blood group determinations include: (i) failure to use sera showing the above properties: (ii) failure to store at low temperature: (iii) pseudo-agglutination or rouleaux formation: (iv) cold agglutination: (v) the Thomsen phenomenon with infected cell suspensions.

(9) The significance of the Rh factor is discussed, and methods of detecting Rh agglutinogens and anti-Rh agglutinins are described.

(10) The necessity of entrusting group determinations only to trained personnel is emphasised.

References

A bibliography covering the recent work on blood grouping would be unduly extensive for a memorandum of this nature. References to the literature concerned with the ABO blood groups may be found in Wiener, A. S. (1939) *Blood Groups and Blood Transfusions*, 2nd edit. London. Publications relating to the Rh factor are listed in Boorman, K. E., Dodd, B. E., and Mollison, P. L. (1942) *Brit. med. J.*, **ii**, 535 and 569.

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